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KNOBBE MARTENS OLSON & BEAR LLP
2040 MAIN STREET
FOURTEENTH FLOOR
IRVINE, CA 92614

EXAMINER	
KIM, ALEXANDER D	

ART UNIT	PAPER NUMBER
1656	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/736,448	LINDEN ET AL.	
	Examiner	Art Unit	
	Alexander D. Kim	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 July 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-54* is/are pending in the application.

4a) Of the above claim(s) *21-54* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-20* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *15 December 2003* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *04/18/2005*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *See Continuation Sheet*.

Continuation of Attachment(s) 6). Other: N-LAUROYLSARCOSINE, Imidazole-Wikipedia, Arginine-Wikipedia and Biotech Dictionary.

DETAILED ACTION

Application Status

1. In response to the previous Office action, a written restriction requirement (mailed on 07/19/2006), Applicants filed a response received on 07/19/2006. Claims 1-54 are pending in this instant Office action.

Election

2. Applicant's election of Group I, Claims 1-20, is acknowledged. Because applicant did not distinctly and specifically point out the status of traverse in the restriction requirement, the election has been treated as an election without traverse (M.P.E.P. § 818.03(a)). The requirement is therefore made FINAL.
Claims 1-54 are pending in the instant application. Claims 21-54 are withdrawn from consideration as non-elected inventions. Claims 1-20 will be examined herein.

Priority

3. The application claims no priority for benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c).

Information Disclosure Statement

4. The information disclosure statement (IDS) filed on 04/18/2005 has been reviewed, and its references have been considered as shown by the Examiner's initials next to each citation on the attached copy.

Objections to the Specification

5. The specification is objected to because of the following informalities:

a. The specification is objected to because the title is not descriptive of the elected claims. A new title is required that is clearly indicative of the invention to which the elected claims are drawn (see M.P.E.P. § 606.01). The examiner suggests the following new title, for example:
---A method for preparing a solution of refolded membrane protein in monodisperse form---.

b. The Abstract is objected to for not completely describing the disclosed subject matter (see M.P.E.P. § 608.01(b)). It is noted that in many databases and in foreign countries, the Abstract is crucial in defining the disclosed subject matter, thus, its completeness is essential. The Examiner suggests the inclusion of the name of the protein(s) (sphingosin 1 phsophate receptor and Cannabinoid receptor 1) for completeness.

c. The "Brief Description of the Drawings" section is missing in the instant specification. Appropriate correction is required.

d. The "Aranti" in p. 24, line 10 should be ---Avanti---. Appropriate correction is required.

e. The "and" or "or" should be placed before "DOPA" in claim 10.

Appropriate correction is required.

Objections to the Drawing

6. The drawings are objected to because examiner cannot decipher what is shown in drawings. It is especially difficult without the Brief Description of drawing in the specification. Corrected drawing sheets in compliance with 37 CFR 1.121(d) are required in reply to the Office action to avoid abandonment of the application. Any amended replacement drawing sheet should include all of the figures appearing on the immediate prior version of the sheet, even if only one figure is being amended. The figure or figure number of an amended drawing should not be labeled as "amended." If a drawing figure is to be canceled, the appropriate figure must be removed from the replacement sheet, and where necessary, the remaining figures must be renumbered and appropriate changes made to the brief description of the several views of the drawings for consistency. Additional replacement sheets may be necessary to show the renumbering of the remaining figures. Each drawing sheet submitted after the filing date of an application must be labeled in the top margin as either "Replacement Sheet" or "New Sheet" pursuant to 37 CFR 1.121(d). If the changes are not accepted by the examiner, the applicant will be notified and informed of any required corrective action in the next Office action. The objection to the drawings will not be held in abeyance.

Claim Objections

7. Claim 10 is objected to because of the following informalities: The conjunction "and" is missing at the end of "sphingomyelin,". Appropriate correction is required.

8. Claims 11 and 16 are objected to because of the following informalities: The comma "," is used instead of the period "." to indicate the decimal point. Appropriate correction is required.

9. Claims 15 and 20 are objected to because of the following informalities: The word "and" is missing at the end of "glucamides;". Appropriate correction is required.

10. Claim 15 is objected to because of the following informalities: The "es-t rs" should be ---esters--- at the end of claim. Appropriate correction is required.

11. Claim 16 is objected to because of the following informalities: The word "or" is missing after the number range "0.05 to 1". Appropriate correction is required.

12. Claim 16 is objected to because of the following informalities: The unit of "% (w/v)" is missing after number ranges of "0.1 to 5" and "0.05 to 1". Appropriate correction is required.

13. Claim 17 is objected to because of the following informalities: The "chromographic" should be ---chromatographic--- in the claim. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

14. Claims 1-20 are rejected under of 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 1 recites the limitation "native" in order to describe the physical state of a membrane protein. However, it is unclear if the claim is limited to a one particular structure among many structures exist in nature because of domain or active site residues movement, for example. Clarification is required.

15. Claims 2 and 10-11 are rejected under of 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 2 recite the limitation "between steps". It is unclear if the claim is intended to put additional step after step (a) limited to a one particular biosynthesis pathway and which enzymes are encompassed by the term. Also, the term between steps is unclear when the claim 1 has no particular order of the method steps. The claim 1 would needs a term "followed by", for example. Clarification is required.

16. Claims 5-6, 8-11, 13, 15-17 and 19 are rejected under of 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Regarding claims 5-6, 8-11, 13, 15-17 and 19, the phrase "preferably" or "especially" renders the claim indefinite because it is unclear whether the limitations following the phrase are part of the claimed invention. See MPEP § 2173.05(d).

17. Claims 4 are rejected under of 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 4 recite the limitation "adequately low concentration". The term "adequately low concentration" in Claim 4 is a relative term, which renders the claim indefinite. The term "adequately low concentration" is not defined by the claims, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. It is unclear how low would meet the scope of the claim. The relative term "an adequately low concentration" without a point of reference and a clear definition makes claims indefinite. Clarification is required.

18. Claim 5 is rejected under of 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 5 recite the limitation "partial", "homologous", "mutated"

and "derived" sequences. The terms "partial", "homologous", "mutated" and "derived" sequences is not defined by the claims, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. It is wholly unclear how related something must be to be considered as "partial", "homologous", "mutated" and "derived" sequence. Clarification is required.

19. Claim 9 is rejected under of 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 9 recites the limitation "the Rapid Translation System (RTS)". There is insufficient antecedent basis for this limitation in the claim which one specifically is intend is unclear. It is unclear if the claims are limited to which one specific cell-free expression system. Clarification is required.

20. Claims 15 and 20 are rejected under of 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 5 recite the limitation "derivatives". The terms "derivatives " is not defined by the claims, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. It is wholly unclear how related something must be to be considered as a "derivatives". Clarification is required.

21. Claim 16 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The word "about" as used in the claim as used to describe the % range of a detergent concentration in a buffer is unclear as to the metes and bounds it imparts on the claimed subject matter. Specifically, it is unclear how varied the percentage can be and still meets the limitation of the claim. Clarification is required.

22. Claim 18 is rejected under of 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 18 recites the limitation "exchanged for". It is unclear if a third detergent is used within step (c) instead of a second detergent or a third detergent is used in additional steps to replace existing the second detergent. Clarification is required.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

23. Claims 1-20 are rejected under 35 U.S.C. § 112, first paragraph, written description, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The instant claims are drawn to a method for preparing a solution for

refolding an eukaryotic membrane protein in monodisperse form by steps comprising adding first detergent, refolding and performing a size exclusion chromatography.

The Court of Appeals for the Federal Circuit has recently held that a "written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as be structure, formula [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." *University of California v. Eli Lilly and Co.*, 1997 U.S. App. LEXIS 18221, at *23, quoting Fiers v. Revel, 25 USPQ2d 1601, 1606 (Fed. Cir. 1993) (bracketed material in original). To fully describe a genus of genetic material, which is a chemical compound, applicants must (1) fully describe at least one species of the claimed genus sufficient to represent said genus whereby a skilled artisan, in view of the prior art, could predict the structure of other species encompassed by the claimed genus and (2) identify the common characteristics of the claimed molecules, e.g., structure, physical and/or chemical characteristics, functional characteristics when coupled with a known or disclosed correlation between function and structure, or a combination of these (paraphrased from *Enzo Biochemical Inc. v. Gen-Probe Inc.* (CAFC (2002) 63 USPQ2d 1609).

University of Rochester v. G.D. Searle & Co. (69 USPQ2d 1886 (2004)) specifically points to the applicability of both *Lily* and *Enzo Biochemical* to methods of using products, wherein said products lack adequate written description. While in *University of Rochester v. G.D. Searle & Co.* the methods were held to lack written description because not a single example of the product used in the claimed methods was described, the same analysis applies wherein the product, used in the claimed

methods, must have adequate written description as noted from *Enzo Biochemical* (see above).

The instant specification claims to teach a method for preparing a solution for refolding a eukaryotic membrane protein by solubilization, refolding and performing a size exclusion chromatography. The breadth of claim includes a genus method for preparing any solution for refolding any membrane protein. Several prior arts teach different methods of solublizing and refolding of eukaryotic membrane proteins and show that all method steps are not same. The specification discloses only one species of method for preparing solutions for refolding two eukaryotic membrane proteins comprising steps of adding a L-lauroyl-sarcosine as the first detergent, refolding of protein in the Ni-NTA affinity column with 1% FOS-choline-14 (C14) as the second detergent and performing a size exclusion chromatography Superdex 200 10/300 GL as disclosed in the Examples. The methods disclosed by the instant specification and the prior art do not sufficiently represent the correlation between the structure and function of claimed genus that is a method for preparing any solutions for refolding any membrane protein, which encompasses species that are widely variant between the species. Thus the disclosure of instant specification and the prior art cannot describe the structure of a very broad genus method and one skilled in the art would not be in possession of the method of claimed genus.

24. Claims 1-20 are rejected under 35 U.S.C. 112, first paragraph, scope of enablement, because the specification, while being enabling for a method for preparing

the solution used in refolding of the protein gpr3 and protein CB1 in the instant application and a method used for certain membrane proteins refolded in a prior art, does not reasonably provide enablement for a method for preparing a solution for refolding any membrane proteins. The specification does not enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and use of the invention commensurate in scope with these claims.

The factors to be considered in determining whether undue experimentation is required are summarized *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). The Court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (Wands, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (Wands, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case are discussed below.

The nature of the invention is drawn to a method for preparing a solution used in refolding steps of eukaryotic membrane protein in monodisperse form and into its native or active form. However, the breadth of claims includes a method for preparing any solution for refolding any eukaryotic membrane protein in monodisperse form by using said refolding steps. Applicants teach a method of preparing a solution used in a refolding steps comprising L-lauroyl-sarcosine as the first detergent, induction of refolding protein in the Ni-NTA affinity column with 1% FOS-choline-14 (C14) as the second detergent and usage of a size exclusion chromatography Superdex 200 10/300 GL or 200 26/60 prep grade. The prior art teaches several method steps including solubilizing, refolding and performing size exclusion chromatography customized for those particular membrane proteins. Applicants disclose no direction or guidance on how to make and use any solutions for any membrane protein that enables a genus method disclosed by the full scope of the claim; i.e. a method of using any buffer, any detergent and any size-exclusion chromatography for refolding any eukaryotic membrane protein in monodisperse form and into its native or active form. Eukaryotic membrane proteins are a genus with very widely variant species thus a membrane protein may irreversibly denatured and cannot be refolded into a native or an active form. The specification and prior art fail to describe how to make and use a method of claimed genus sufficiently by the instant disclosure. Therefore, it is unpredictable for a method for preparing any solution to be used in said method steps of refolding any eukaryotic membrane protein. For all of the above reason, it would require undue

experimentation necessary for a said method steps of refolding any eukaryotic membrane protein.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

25. Claims 1-5, 7-8, 10, 12-13 and 15-17 are rejected under 35 U.S.C. 102(b) as being anticipated by reference by Dutta et al. (2002, Infection and Immunity, Vol. 70, p. 3101-3110) as evidenced by Raetz et al. (1990, The Journal of Biological Chemistry, Vol. 265, p. 1235-1238). Claim 1 (dependent claims 2-8, 10, 12-13 and 15-~~20~~ 17 therefrom) is drawn to a method for preparing a solution of refolded, recombinantly expressed eukaryotic membrane protein in monodisperse form, comprising the steps of solubilize in a first detergent, refolding into its native or active form and performing a size exclusion chromatography on a refolded membrane protein with additional limitations in dependent claims.

Dutta et al. teach a method for preparing a recombinant *Plasmodium falciparum* (a protozoan parasite) apical membrane antigen 1 (AMA1), which is an "integral membrane protein" (see bottom of left column, p. 3101). The recombinantly expressed protein with His-tag was designated as r-AMA1/E (bottom right column, p. 3101). The E. coli cell pellet was resuspended and "A solution of 20% sodium N-lauroylsarcosine

(Sarkosyl) was added to a final concentration of 5% detergent", as a first detergent (see bottom left column, p. 3102). The r-AMA1/E was refolded in the presence of glutathione with or within Ni2+ column as shown in the Refolding (top right column, p. 3102). The "biochemical characterization and evidence of correct folding of AMA1/E are presented" (p. 3102, left column, lines 2-4). "A single peak was shown" (right column, p. 3105, lines 5-6) from the "gel permeation Shodex Protein KW-803 column elution profile" (Fig. 2 description) thus meets the limitation of protein in monodisperse form from a size exclusion column. The r-AMA1/E meets all the limitations of Claim 1, 5, 7 and 12 as described above. The naturally occurring E. coli phospholipids including "phosphatidylcholine", "phosphatidylinositol", "sphingomyelin" and "glycerophosphlipid" (last line of left column-2nd line of right column, p. 1235) as disclosed by Raetz et al. (1990) was inherently added by detergent solublization of cell extract thus meets the limitation of claim 2 and 10. The first detergent Sarkosyl was exchanged with 500 mM imidazole (a second detergent with the broadest reasonable definition of detergent which has both hydrophilic and hydrophobic parts, see copy of Biotech Dictionary in the attachment) during the Ni^{2+} column purification and the final concentration of the first detergent Sarcosyl was lowered to 0.125 % by "elution buffer D" (bottom left column, p. 3102) thus meets the limitations of claims 3-4, 15 and 17. The "protein fractionation experiments showed that the majority of r-AMA1/E was still localized in the insoluble fraction" thus meets the limitation of claim 8. The specific gravity of Sarcosyl is 0.97-0.99 (see Sarcosyl L in the attachment) thus 5% would be 4.85-4.95 % (W/V) thus meets the limitation of claim 13. 25.04 g of imidazole, the second detergent with MW of

68.08, is needed to make 500 mM in Buffer D which is 2.5 % (w/v) thus meets the limitation of claim 16. Thus, Dutta et al. teach a method which meets all limitations of claims 1-8, 10, 12-13 and 15-17.

26. Claims 1-8, 10 and 18 are rejected under 35 U.S.C. 102(b) as being anticipated by reference by Zhang et al. (2002, Protein Expression and Purification, Vol. 25, p. 105-113). Claims 1-8, 10 and 18 are drawn to a method for preparing a solution of refolded, recombinantly expressed eukaryotic membrane protein in monodisperse form, comprising the steps of solubilizing in a detergent, refolding into its native or active form and performing a size exclusion chromatography on a refolded membrane protein.

Zhang et al. teach a method for preparing a B7-2 which is a "glycosylated type I transmembrane proteins" (see top left column, p. 106). Zhang et al. "established a bacterial expression system for the production of recombinant human B7-2 for biochemical and X-ray crystallographic studies" (see bottom right column, p. 106). The "hB7-2V, expressed as inclusion bodies", were dissolved in buffer containing 6M guanidine-hydrochloride (Gu-HCl, as a first detergent) as described in the middle of left column, p. 107. Sixteen milligrams of solubilized inclusion bodies were diluted into a buffer composed of 10 mM NaAc, 6M Gu-HCl (as a second detergent), 5mM EDTA and pH 4.6, and the protein was refolded by rapid dilution over few seconds into 1 liter of refolding buffer having 0.4 M arginine-HCl (see middle of left column, p. 107) as third detergent because it is "considered to be an amphipathic" (see Arginine-Wikipedia in the attachment). Zhang et al. also teach a method step of loading refolded protein

solution into a "gel-filtration chromatography using a Superdex G-75 column" (bottom left column, p. 107) and "revealed a monodisperse peak at ~20 min" (see last line of right column, p. 108) and as shown in Figure 2A, p. 109. The refolding of hB7-2V into its native form was evidenced by the binding assay result by "incubation of hB7-2V and monomeric CTLA-4 at a 1:1 molar ratio overnight at 4oC, both gel-filtration chromatography and native PAGE demonstrated the formation of a distinct species corresponding to the monomeric CTLA-4/B7-2 complex" (see bottom right column, p. 109). The inclusion bodies recovered by the centrifugation of french pressured cell extract contains naturally occurring phospholipids thus meets the claim limitation of 2 and 10. Thus, Zhang et al. teach a method that meet all the limitation of the instant claims 1-8, 10 and 18

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

27. Claims 1-5, 7-9, 10 and 14-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chang et al. (2001, Protein Expression and Purification, Vol. 23, p. 432-439) in view of Kiefer (2003, Feb. 17, Biochemimica et Biophysica Acta, Vol 1610, p. 57-62, in the IDS) and further in view of Winzor (2003, June 30th, J. Biochem. Biophys. Methods, Vol. 56, p 15-52).

Chang et al. teach a method for preparing a solution for refolding of "expressed recombinant yeast DDPPs using Escherichia coli as the host cell" (see middle of left column, p. 432). The DDPPs of Chang et al. is "found to be primarily membrane bound in the yeast" (see top left column, p. 433). Chang et al. use pelleted DDPPs from inclusion bodies from the E. coli expression system and dissolved in 7 M urea (a first detergent), exchanging the first detergent by dialyzing against 10 volume of a buffer with 6mM β-mercaptoethanol (a second detergent which is substance with both hydrophobic and hydrophilic part, see copy of Biotech Dictionary in the attachment) and refolded in a buffer with "0.1% Triton X-100 or 0.05% n-octyl-β-D-glucopyranoside" as a third detergent (see top right column, p. 434). Chang et al. also teach a method of refolding the protein on the Ni-NTA column by binding a His tagged DDPPs onto the Ni-NTA column and exchange Urea buffer with the 0.1% Triton X-100 (a third detergent) (see top right column, p. 434). Finally, the thioredoxin and His tag located in the N-terminal of DDPPs were removed and further purified using streptavidin-agarose and Ni-NTA (see middle of right column, p. 434). Chang et al. also "confirm that the recombinant DDPPs after refolding was active" and kinetic constant were determined as disclosed in bottom right column, p. 437. The purified DDPPs of Chang et al. is shown in SDS/PAGE gel of Figure 3 (p. 437) meets the limitation of proteins in monodisperse form as cited in the claim 1 with a broadest reasonable interpretation (see definition of monodisperse in Britannica Online in the attachment).

Chang et al. do not teach a method of performing a size exclusion chromatography.

Chang et al. do not teach a method of adding lipids before refolding steps.

Chang et al. do not teach a method of using cell-free expression system for making membrane protein.

Winzor (2003) teach a method of isolating a molecules using size exclusion chromatography to create monodispersed protein.

Kiefer (2003) teach a method of adding an "E. coli phospholipid" (see middle right column, p. 58) before refolding, a method of making membrane protein aggregate by cell-free synthesis and a method of using cell-free synthesis for making membrane protein (top right column, p. 60).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the claimed invention was made by a method of isolating a refolded protein DDPPs of Chang et al. and use a method of size exclusion chromatography suggested by Winzor (2003) with a reasonable expectation of success of isolating DDPPs in order to form proteins in monodisperse state because the size exclusion chromatography fractionate "protein mixture on the bases of molecular size" (see line 1-2 in Introduction of Winzor et al.). The motivation to do so is provided by Winzor (2003) who teaches the usefulness of using size exclusion chromatography having advantages of "(1) the rapidity of partition equilibrium attainment between mobile and stationary phases and (2) the relative insensitivity of chromatographic behavior to solute concentration" (see p. 15, last line - p. 16, line 2).

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform a method of isolating a refolded protein DDPPs

of Chang et al. and use a method of adding a phospholipid before refolding and/or use a method of cell-free synthesis for making membrane proteins of Kiefer (2003) with a reasonable expectation of success to make and refold DDPPs of Chang et al. because the "Mixtures of lipids and detergents, so called mixed micelles and bicelles, are probably better solubilizing agents for inclusion bodies" (see top right column, p. 58). The motivation to do so is provided by Kiefer (2003) who teaches the usefulness of refolding a membrane protein which "mediate flow of information or substances between the cytosol and extracellular space" (middle left column, p. 57, line 3-5). Kiefer (2003) teaches a method of using a phosphatidylethanolamine and other phospholipids (see top right column, p. 58) as a lipid.

Also, It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform a method of refolding protein DDPPs of Chang et al. with a method of using the cell free expression of membrane proteins leading to aggregation with a reasonable expectation of success to make and refold DDPPs of Chang et al. because more protein is made by avoiding the interference of detergent in the transcription/translation in-vitro translation (see middle right column, p. 58, Kiefer 2003). The motivation to use a cell-free synthesis of membrane protein is provided by Kiefer (2003) who teach the usefulness of cell-free synthesis because it yields "higher quantities" and avoid interference of the transcription/translation system from the added detergent (see top right column, p. 60, lines 7-19). Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

28. Claims 1-6, 8-10, 12, 13 and15-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Jekabsons et al. (2002, Biochem. J., Vol 366, p. 565-571) and Stuart et al. (2001, The Journal of Biological Chemistry, Vol. 276, p. 18633-18639) in view of Winzor (2003, June 30th, J. Biochem. Biophys. Methods, Vol. 56, p 15-52) and in further view of Kiefer (2003, Feb. 17, Biochemimica et Biophysica Acta, Vol 1610, p. 57-62).

Jekabsons et al. teach a method for preparing a solution for refolding of "recombinant human uncoupling protein-2 (UCP2)" (see Abstract, 2nd line). The UCP2 of is over-expressed as an inclusion body from a E. coli, which is described in Stuart et al. (2001) as cited in Preparation of UCP2 inclusion bodies (see top left column, p. 566). Jekabsons et al. also teach the "UCP-2 is a mitochondrial inner membrane protein" and activity of binding nucleotide (see 2nd paragraph, left column, p. 565). The method of Jekabsons et al. use 1.5% sarkosyl, as a first detergent, to solubilize UCP2 in the form of inclusion bodies (see p. 566, left column, line 8). The induction of UCP2 refolding was performed during detergent exchange by "removal of sarkosyl and the addition of non-ionic detergent (C8E5, C10E6, C12E9, C13E10 or digitonin)" (see p. 566, left column, lines 1-2 in the Detergent exchange). The final concentration of the second detergent was 0.1-1% (v/v), which is 0.1-1% (w/v) when the density of buffer is about 1 mg/ml. The sample containing refolded UCP2 were further purified by Hydorxyapatite column chromatography. The purified UCP2 shown in SDS/PAGE of Figure 2 (p. 567) meets the limitation of protein in monodisperse form within the broadest reasonable interpretation. The binding of nucleotide of refolded UCP2 as shown in Table 2, p. 569 teach that the refolded UCP2 has a native form or an active form.

Jekabsons et al. do not teach a method of performing a size exclusion chromatography.

Jekabson et al. no not teach a method of adding a lipid in between the step of using first and second detergent.

Jekabson et al. do not teach a method of using cell-free expression system for making membrane protein.

Winzor (2003) teach a process of isolating a molecules using size exclusion chromatography.

Kiefer (2003) teach a method of adding a lipid after a first detergent solubilization and a method of making membrane protein aggregate by cell-free synthesis and a method of using cell-free synthesis for making membrane protein.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made by a method of isolating a refolded protein UCP2 of Jekabsons et al. and use a method of size exclusion chromatography suggested by Winzor (2003) with a reasonable expectation of success of isolating UCP2 because the size exclusion chromatography fractionate "protein mixture on the bases of molecular size" (see line 1-2 in Introduction of Winzor et al.). The motivation to do so is provided by Winzor (2003) who teaches the usefulness of using size exclusion chromatography having advantages of "(1) the rapidity of partition equilibrium attainment between mobile and stationary phases and (2) the relative insensitivity of chromatographic behavior to solute concentration" (see p. 15, last line - p. 16, line 2).

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made by a method of refolding protein UCP2 of Jekabsons et al. and use a method of adding a lipid after a first detergent solubilization and use a method of cell-free synthesis for making membrane proteins of Kiefer et al. with a reasonable expectation of success to make and refold UCP2 of Jekabsons et al because the "mixtures of lipids and detergents, so called mixed micelles and bicelles, are probably better solubilizing agents for inclusion bodies" (see top right column, p. 58). The motivation to do so is provided by Kiefer (2003) who teaches the usefulness of refolding a membrane protein which "mediate flow of information or substances between the cytosol and extracellular space" (middle left column, p. 57, line 3-5). Kiefer teaches a method of using a phosphatidylethanolamine and other phospholipids (see top right column, p. 58) as a lipid.

It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to perform a method of refolding protein DDPPs of Chang et al. with a method of using the cell free expression of membrane proteins leading to aggregation with a reasonable expectation of success to make and refold DDPPs of Chang et al. because more protein is made by avoiding the interference of detergent in the transcription/translation in-vitro translation (see middle right column, p. 58, Kiefer 2003). The motivation to use a cell-free synthesis of membrane protein is provided by Kiefer (2003) who teach the usefulness of cell-free synthesis because it yields "higher quantities" and avoid interference of the transcription/translation system from the added

detergent (see top right column, p. 60, lines 7-19). Thus, the claimed invention as a whole was *prima facie* obvious over the combined teachings of the prior art.

Conclusion

29. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Alexander D. Kim whose telephone number is (571) 272-5266. The examiner can normally be reached on 8AM-5PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen Kerr can be reached on (571) 272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Alexander Kim
21 August 2006

KATHLEEN M. KERR, PH.D.
SUPERVISORY PATENT EXAMINER